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# Combination of Ki67 Proliferation Index and CD10 in Prognosis of Patients with Follicular Lymphoma

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## Abstract

**Background &Objective:** Markers such as Ki67 and CD10 play a role in the prognosis of follicular lymphoma (FL). However, the combined effect of these factors is still unclear. Our objective was to determine the combination of Ki67 and CD10 in the prognosis in patients with FL.

**Method:** Twenty-seven patients with FL were retrospectively analyzed. Based on immune histochemical staining for Ki67 and CD10 that was performed in biopsy lymph node, the patients were grouped according to the levels of the Ki67 proliferation index (PI) and the presence of CD10. Univariate and multivariate analysis was performed according to Ki67 proliferation index (PI) levels and CD10 presence.

**Results:** The ROC curve (receiver operating characteristic) found that the cut-off point of 60% for Ki67 was statistically significant in the difference in survival rates. Multivariate analysis suggested that Ki67>60%, CD10 negative was a truly independent prognostic factor for progression- free survival (PFS) (*P*= 0.045 HR=4.817;*P*=0.038 HR=5.195, respectively). There was a statistically significant difference between the groups: CD10 positive/Ki67≤60%, CD10 positive/Ki67>60%, CD10 negative/Ki67≤60%, and CD10 negative/Ki67>60% (*P*=0.007). Patients with positive CD10/Ki67≤60% had the best PFS. Patients with negative CD10 /Ki67>60% had the worst PFS.

**Conclusion:** Ki67>60%, CD10 negative are truly independent adverse prognostic factors for PFS in FL. Patients with CD10 negative/Ki67>60% had worst PFS.

## Keywords

Ki67 proliferation index; CD10; Follicular lymphoma; FL; Prognosis.

## Introduction

Follicular lymphoma (FL) is an indolent lymphoma and is associated with a long survival time (OS). However, many patients relapse and have short progression- free survival (PFS) [1,2]. There are common prognostic systems: FLIPI (Follicular Lymphoma International Prognostic Index), FLIPI2, m7-FLIPI [1]. These systems used clinical factors, laboratory factors, and genes in combination to calculate the prognostic score. However, these prognostic systems have limitations, especially in the development of new therapeutic methods [1,3,4]. Therefore, more risk factors are still being studied; especially there are many studies that pay attention to the use of markers. They are necessary for diagnosis and have prognostic significance [5,6].

Ki67 is a marker of cell proliferation with the controversial effect on FL. There are not many studies that examine the role of Ki67 in slow-growing diseases such as FL. Kawaguchi Y, et al. showed that the patients with high expression of Ki67 seem to have had worse OS [7]. But Xue T, et al. showed that patients with a higher Ki67 index had better PFS [8].

CD10 is a cell membrane metallopeptidase that is widely distributed on neoplastic cells in FL. It can be considered as a surrogate marker for a slow-growing disease such as FL. The appearance of loss of CD10 expression can be seen as a signal of a transformation that progresses. Chen SW et al. showed that loss of CD10 expression was related to leukemia transformation [9]. Camacho FI et al. suggested that strong positive CD10 was a favorable factor OS, but did not show how it was strong positive [10]. Bilalovic N et al. suggested that CD10 positive patients would have a longer OS [11]. However, there are almost no studies showing that the combination of Ki67 and CD10 has an effect on FL. Our aim was to determine the combination of Ki67 and CD10 in the prognosis in the patient with FL.

## Methods

### Patients

This study was carried out in Bach Mai Hospital, Hanoi, Vietnam. Twenty-seven patients, from March 2016 to July 2021, with de novo FL were retrospectively analyzed in our study. All patients were diagnosed by examination of lymph node biopsies based on H.E staining and immunohistochemical staining for CD20, CD10, CD3, CD5, CD23, Bcl2, Bcl6, MUM1 and Ki67. The diagnosis was made according

to the WHO 2008 classification of hematopoietic and lymphoid tumors [12,13]. The patients were treated with the R-CHOP protocol (rituximab- cyclophosphamide, doxorubicin, vincristine, prednisolone) or the R-COP protocol (rituximab- cyclophosphamide, vincristine, prednisolone). The response to therapy was determined according to the criteria of the International Working Group (RECIL 2017) [14].

### Definition

CD10 had been scored as 'positive' when at least 30% of the cells showed expression [15].

### Statistics

The ROC curve (receiver operating characteristic) was performed separately for Ki67 level to gain a predictive value for OS. This found cut-off was applied for PFS to determine if there was statistically significant difference in survival rate. The patients were then grouped according to the newly found Ki67 PI cut-off value. The patients were also grouped according to the presence of CD10.

Independent-sample T tests were used to analyze differences in quantitative variables between the groups of patients. The $\chi^2$ or Fisher's exact tests were used to analyze differences in qualitative variables between the groups of patients.

The Kaplan-Meier method was used to analyze OS and PFS.

Univariate analysis (using the log-rank test) and multivariate analysis (using the Cox proportional hazards method) with the Ki67 and CD10 variables were performed to determine prognostic factors for OS and PFS.

## Results

### Patients characteristics

Table 1 shows that there were no statistically significant differences in laboratory indices between the two groups (Ki67≤60% vs. Ki67>60%, CD10 positive vs. CD10 negative).

| | Ki67 | N | Mean | *P* | CD10 | N | Mean | *P* |
|---|---|---|---|---|---|---|---|---|
| **Age (years)** | ≤60% | 23 | 58.3043 | >0.05 | positive | 17 | 56.8235 | >0.05 |
| | >60% | 4 | 64.5000 | | negative | 10 | 63.3000 | |
| **Hemoglobin (g/L)** | ≤60% | 23 | 130.5217 | >0.05 | positive | 17 | 133.2941 | >0.05 |
| | >60% | 4 | 136.5000 | | negative | 10 | 128.2000 | |
| **Platelet (x$10^9$/L)** | ≤60% | 23 | 227.4348 | >0.05 | positive | 17 | 224.3529 | >0.05 |
| | >60% | 4 | 322.0000 | | negative | 10 | 270.5000 | |
| **WBC (x$10^9$/L)** | ≤60% | 23 | 11.7087 | >0.05 | positive | 17 | 12.7665 | >0.05 |
| | >60% | 4 | 7.6875 | | negative | 10 | 8.3020 | |
| **LDH (U/L)** | ≤60% | 23 | 260.1739 | >0.05 | positive | 17 | 271.5294 | >0.05 |
| | .60% | 4 | 241.0000 | | negative | 10 | 233.2000 | |
| **AST (U/L)** | ≤60% | 23 | 26.0435 | >0.05 | positive | 17 | 24.8824 | >0.05 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | >60% | 4 | 23.0000 | | negative | 10 | 26.8000 | |
| **ALT (U/L)** | ≤60% | 23 | 24.0000 | >0.05 | positive | 17 | 25.5294 | >0.05 |
| | >60% | 4 | 22.7500 | | negative | 10 | 20.9000 | |
| **Albumin (g/L)** | ≤60% | 23 | 39.5739 | >0.05 | positive | 17 | 40.7529 | >0.05 |
| | >60% | 4 | 37.0250 | | negative | 10 | 36.5500 | |
| **Bilirubin (µmol/L)** | ≤60% | 23 | 8.0783 | >0.05 | positive | 17 | 7.9765 | >0.05 |
| | .60% | 4 | 6.4000 | | negative | 10 | 7.5800 | |
| **Ure (mmol/L)** | ≤60% | 23 | 5.7261 | >0.05 | positive | 17 | 5.7118 | >0.05 |
| | >60% | 4 | 4.9500 | | negative | 10 | 5.4400 | |
| **Creatinin (mmol/L)** | ≤60% | 23 | 62.2783 | >0.05 | positive | 17 | 61.0824 | >0.05 |
| | >60% | 4 | 59.7500 | | negative | 10 | 63.3000 | |

**Table 1:** Patients characteristics according to Ki67 and CD10.

There were also no significant differences in clinical indices (FLIPI, bone marrow involvement, hepatosplenomegaly, B syndromes, high tumor burden, Ann Arbor stage) between the two groups (Ki67≤60% *vs.* Ki67>60%, CD10 positive *vs.* CD10 negative) (Table 2).

| | | **Ki67** | | ***P*** | **CD10** | | ***P*** |
|---|---|---|---|---|---|---|---|
| **Clinical factors** | | **Ki67≤60% (n=23)** | **Ki67>60% (n=4)** | | **CD10 positive (n=17)** | **CD10 negative (n=10)** | |
| **Ann Arbor Stage** | IIB | 1 | 0 | >0.05 | 1 | 0 | >0.05 |
| | IIIA | 1 | 0 | | 1 | 0 | |
| | IIIB | 14 | 4 | | 9 | 9 | |
| | IVB | 7 | 0 | | 6 | 1 | |
| | Total | 23 | 4 | | 17 | 10 | |
| **Bone Marrow Involvement** | No | 18 | 4 | >0.05 | 13 | 9 | >0.05 |
| | Yes | 5 | 0 | | 4 | 1 | |
| | Total | 23 | 4 | | 17 | 10 | |
| **Hepatosplenomegaly** | No | 16 | 4 | >0.05 | 12 | 8 | >0.05 |
| | Yes | 7 | 0 | | 5 | 2 | |
| | Total | 23 | 4 | | 17 | 10 | |
| **B Syndrome** | No | 1 | 0 | >0.05 | 1 | 0 | >0.05 |
| | Yes | 22 | 4 | | 16 | 10 | |
| | Total | 23 | 4 | | 17 | 10 | |
| **High Tumor Burden** | No | 0 | 0 | >0.05 | 0 | 0 | >0.05 |
| | Yes | 23 | 4 | | 17 | 10 | |
| | Total | 23 | 4 | | 17 | 10 | |
| **FLIPI** | Low | 1 | 0 | >0.05 | 1 | 0 | >0.05 |
| | Inter | 6 | 2 | | 5 | 3 | |
| | High | 16 | 2 | | 11 | 7 | |
| | Total | 23 | 4 | | 17 | 10 | |

**Table 2:** Clinicopathological profile of patients.

## Ki67 and CD10 in survival times (OS and PFS)

The ROC curve found that the cut-off point of 60% for Ki67 was statistically significant in the difference in OS (AUC=0.98, sensitivity: 100%, specificity: 90%,*P*=0.026), (Figure1). This cut-off was applied for PFS and it was determined that there was statistically significant difference in survival rate. In univariate analysis, OS and PFS in the Ki67 PI>60% group had decreased statistically significantly for 5 years, OS and PFS in the CD10 negative group also had decreased statistically significantly for 5 years, (Table 3).

Multivariate analysis showed that the Ki67>60%, CD10 negative was a truly independent adverse prognostic factor for PFS (*P*= 0.045, 0.038; respectively) (Table 3).

Table 4 and Figure 2 show that there was a statistically significant difference in PFS between the groups: CD10 positive/Ki67≤60%, CD10 positive/Ki67>60%, CD10 negative/Ki67≤60% and CD10 negative/Ki67>60% (*P*=0.007). Patients with positive CD10/Ki67≤60% had the best PFS. Patients with negative CD10/Ki67>60% had the worst PFS.

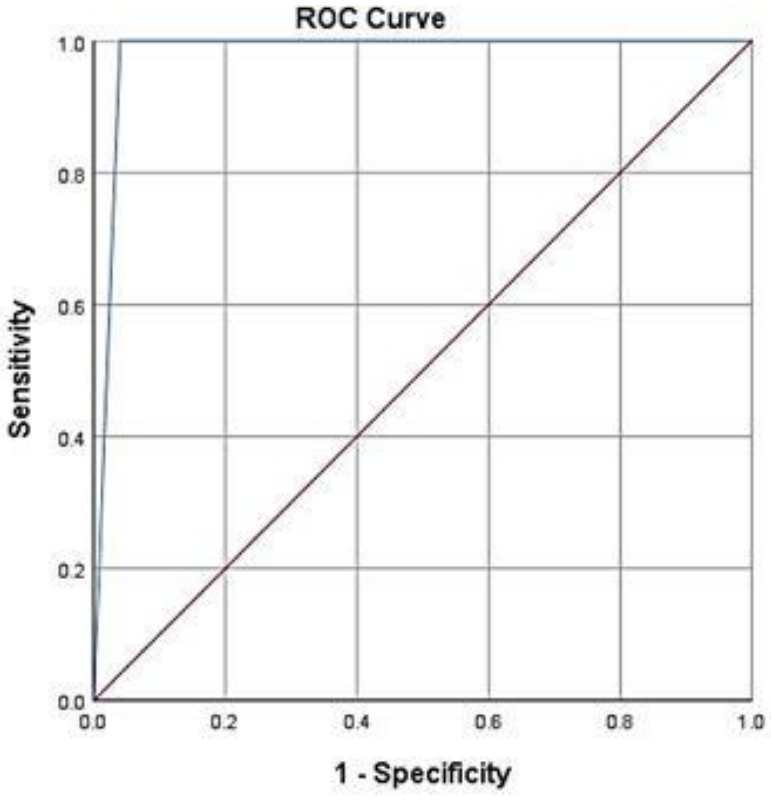


**Figure 1:** ROC (receiver operating characteristic) curve and area under curve for Ki67 percent (AUC=0.98, sensitivity: 100%, specificity: 90%, *P*=0.026).

| Factor | Univariate analysis (OS) | Multivariate analysis (OS) | | |
|---|---|---|---|---|
| | $P^{\text{Log-rank}}$ value | HR | 95%CI | $P^{\text{Cox}}$ value |
| **Ki67** | | | | |
| ≤60% | 0.000 | | | >0.05 |
| >60% | | | | |
| **CD10** | | | | |
| Positive | 0.049 | | | >0.05 |
| Negative | | | | |
| **Factor** | **Univariate analysis (PFS)** | **Multivariate analysis (PFS)** | | |
| | $P^{\text{Log-rank}}$ value | HR | 95%CI | $P^{\text{Cox}}$ value |
| **Ki67** | | | | |
| ≤60% | 0.016 | 1 | | 0.045 |
| >60% | | 4.817 | 1.034-22.454 | |
| **CD10** | | | | |
| Positive | 0.017 | 1 | | 0.038 |
| Negative | | 5.195 | 1.094-24.666 | |

**Table 3:** Univariate and multivariate analysis for survival times.

| Factors | PFS (months) | *p* |
|---|---|---|
| CD10 positive/ Ki67≤60% | 45.696 | 0.007 |
| CD10 positive/ Ki67>60% | 29.000 | |
| CD10 negative /Ki67≤60% | 27.729 | |
| CD10 negative /Ki67>60% | 3.333 | |

**Table 4:** Combination of Ki67 and CD10 in prognosis for progression free survival.

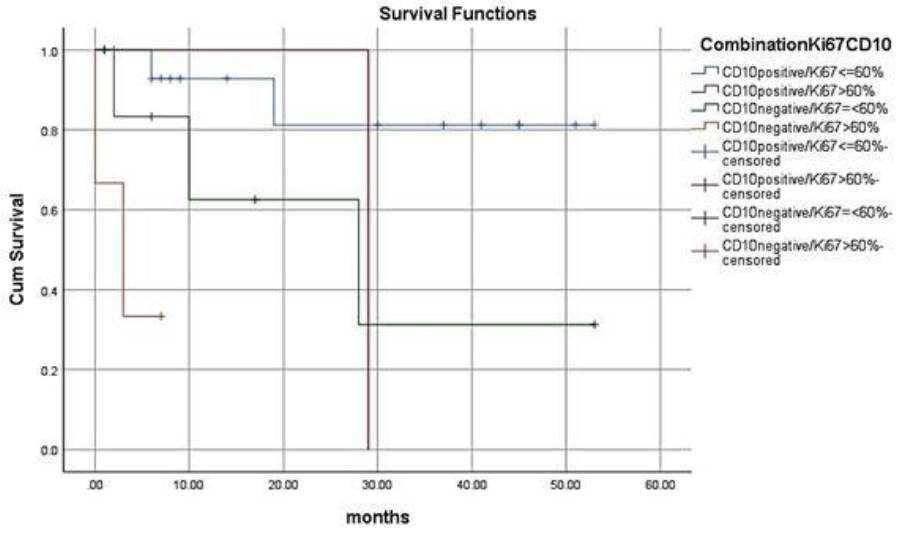


**Figure 2:** Progression free survival according to the combination of Ki67 and CD10.

## Discussion

Unlike DLBCL (diffuse large B cell lymphoma) or MCL (mantle cell lymphoma) [16,17], the prognostic value of Ki67 in Fl is controversial. In some univariate analyzes, high expression of Ki67 appears to be a significant adverse factor, as in a study by Kawaguchi Y et al. or in a study by Xerri L et al. [7,18]. However, in multivariate analysis, these studies have not shown a statistically significant difference. Llanos M et al. also showed the same result [19]. Furthermore, Camacho FI et al. did not observe any differences in OS between patients who were grouped by Ki67 expression [10]. But Xue et al. showed the surprising conclusion that a higher Ki67 was a favorable factor for PFS [8]. In the univariate analysis, our study showed that at a high expression level (>60%), Ki67 has an adverse effect on OS and PFS. But in the multivariate analysis, high expression of Ki67 has only an adverse effect on PFS. Ki67 is a proliferation antigen, so it is generally an adverse factor in isolation. However, when expressed in a slow-growing disease such as FL and considered with other factors, the analysis becomes more difficult.

Unlike Ki67, the favorable role of CD10 in FL appears to be more consensual. In univariate and multivariate analysis, Camacho FI et al., Bilalovic N et al., both suggested that CD10 expression was related to significantly better OS [10,11]. However, when multivariate analysis was performed, our study showed that the CD10 negative was a truly independent adverse prognostic factor only for PFS, not for OS.

When evaluating the association between Ki67 and CD10 in the effect on survival time, our study showed that there was a statistically significant difference in PFS between the groups: CD10 positive/Ki67≤60%, CD10 positive/Ki67>60%, CD10 negative/Ki67≤60%, and CD10 negative/Ki67>60% (*P*=0.007). Patients with positive CD10/Ki67≤60% had the best PFS. Patients with negative CD10 /Ki67>60% had the worst PFS. In contrast, in multivariate analysis, Camacho FI, et al. suggested that

CD10 was a favorable factor for OS, while Ki67 was not significant [10]. However, this research was based on a group treated with a regimen without rituximab, while in our study, all patients were treated with a protocol containing rituximab. This result is completely consistent with the idea that, in the era of rituximab, PFS was used to assess the outcome of follicular lymphoma rather than OS.

Our study has some limitations, as there are patients with symptomatic disease or with a high tumor burden, who are indicated for chemotherapy. However, FL is an indolent lymphoma, so there is a not small number of patients without symptomatic disease or with low tumor burden, who are indicated for observation. Therefore, this study has not yet covered all patients with FL and should continue to be conducted with a large number of patients, with indications for chemotherapy and observational groups.

## Conclusion

Ki67>60%, CD10 negative are truly independent adverse prognostic factors in FL for PFS. Patients with CD10 negative/Ki67>60% had worst PFS.

## Ethics

The study protocol was approved by the Ethics Committee. The patient's consent was waived by the committee, as this study was a retrospective observational study.

## Financial Disclosure Statement

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## Conflicts of Interest/Funding

The authors declare no conflicts of interest.

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